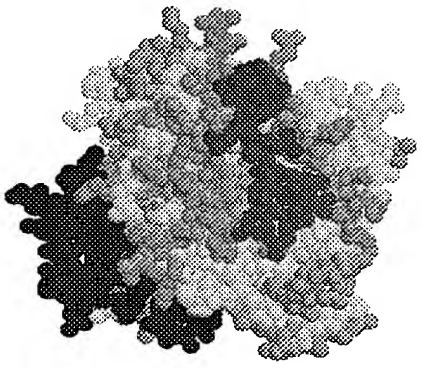
Thrombin

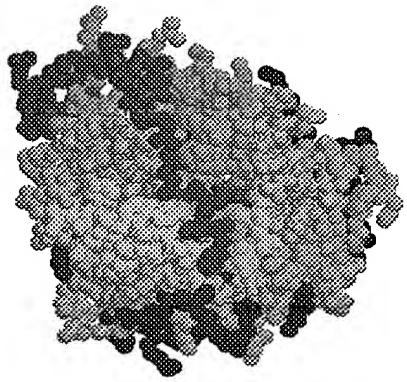
Thrombin is a member of a family of glycoproteins that are involved in the process of blood clotting. The process of blood clotting involves a large cascade of enzymes, factors and similar derivatives. Thrombin is involved in the latter stages of this process specifically in interactions between heparin and several blood clotting factors which include Factor V, VIII and XIII resulting in the proteolysis of fibrinogen to fibrin. It possesses two main binding sites one is a catalytic binding site and the second is a binding site that recognises heparin. The molecule as a whole shows great similarity in structure to the serine proteases such as trypsin and chymotrypsin including a hydrophobic cleft for the 'docking' of the substrate. During the blood clotting cascade it exhibits great specificity in its action by only cleaving a limited number of bond types. These are mainly Arg-Gly.

As its function suggests, thrombin is required in circulation in the blood. This means that it readily comes into contact with water. As it forms part of a cascade of enzymes its main method of regulation is by the presence of a substrate to product ratio. The whole process of blood clotting is originally initiated by the immune response in reaction to injury. Thrombin can also be regulated by the presence of inactivators. It can be inactivated by many different inhibitors including antithrombin III, but one of the main naturally occurring inhibitors is hirudin. Hirudin is a small protein that is found in the European medicinal leech that binds into the catalytic site of the enzyme.

It is very difficult to obtain a crystallograph of thrombin alone and due to this the structure that is seen below is an α -thrombin molecule in a complex with hirudin. The illustration below shows the whole molecule which consists of two peptide sub-chains. The larger of the two chains is illustrated as green consisting of 259 residues and is termed the heavy chain (H). The shorter of the two chains is illustrated in blue and comprises of 36 residues. This is called the light chain (L). The two chains are linked together by disulphide bonds to form the complete molecule. Shown in red on the illustration below is the 65 residue hirudin chain. Also illustrated below is the hydrophobic core of the molecule, hydrophobic regions being shown in yellow, which is consistent with the molecule being found free within the blood. There are also many interactions with water on the surface of the molecule although these are not shown.

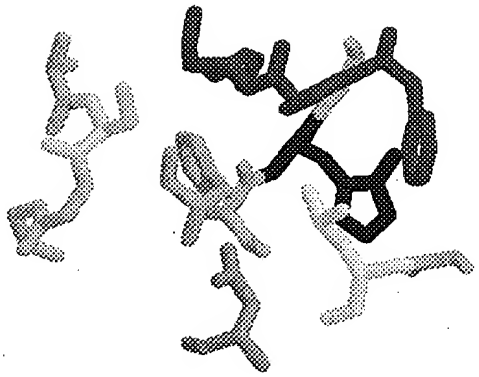


The molecule has two main binding sites. The active site that is bound by hirudin is shown below in light blue. The second binding site, shown here in purple, is thought to be the binding site of heparin. This area is highly charged forming an anion-recognition binding site.



The structure within the protein consists mainly of β sheets, shown in yellow, although there are a few helices, shown in red. The β sheets appear to form two barrel-like structures. In the illustration below there is one barrel to the left which is seen sideways on and one to the right which is viewed from above. The hirudin is shown in green and the tail can be seen sitting in a groove over the latter of the two barrels. One of the main structural differences between this and other serine proteases is that it has additional loop structures. (Loops shown in blue.)

When looking at the binding site between hirudin and thrombin in more detail it can be seen that the N-terminal of the hirudin, shown in red, sits within a hydrophobic pocket. The illustration below shows the other interacting regions within the binding site area. The closest bonds form between hirudin (red) and Ser 195 (yellow) on the H chain of thrombin. Here the atoms are within hydrogen bonding distance. His 57 (cyan) and Asp 102 (green) also form important binding interactions. Leu 99 (white) and His 57 are also important in forming non-polar contacts with the hydrophobic 'docking area' of the hirudin N-terminal. The hydrophobic cleft itself is formed by Ile 174 and Trp 215 (blue).



Comments on these pages are welcome and may be mailed to a.thomson@lancaster.ac.uk.
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